The Structure and Post-Embryonic Development of the Alimentary Canal of the Mustard Sawfly, Athalia proxima Klug. (Tenthredinidae, Hymenoptera)

Dissertation submitted in partial fulfilment
for the Degree of Master of Philosophy
IN
ZOOLOGY
OF
The Aligarh Muslim University, Aligarh

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D E D I C A T E D

TO

MY MOTHER

IN

GRATITUDE

I certify that the contents of this dissertation entitled "The Structure and Post-embryonic Development of the Mustard Sawfly, Athalia proxima Klug. (Tenthredinidae, Hymenoptera)" is the original work of Miss Suhela Nayab done under my supervision. This work is hereby submitted in partial fulfilment of the requirements for the award of the degree of Master Of Philosophy of the Aligarh Muslim University, Aligarh.

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C O N T E N T S

I. INTRODUCTION

Much work has been done on the Embryology of several orders of insects. Among Hymenoptera mostly the honey-bee has been a subject of wide investigations. Nelson (1915) has worked out thoroughly the embryonic development of the honey-bee and has also given a brief account of the first instar larva. Oertel (1930) described its metamorphosis.

Among the lower Hymenoptera the family Tenthredinidae comprises a group of insects which are primitive in comparison to the higher Hymenoptera. The selection of the mustard sawfly, Athalia proxima Klug., for the study of its post-embryonic development is primarily based on the fact that since its morphology and Embryology has already been worked out leaving alone studies on its postembryology. This work it is hoped, when completed, might fill up the inevitable gap. It would then be possible to get a fuller account of the development of one species of the family Tenthredinidae. Such an information would go a long way to give us an idea of such organs which persist throughout life from the embryo to the adult. Those which modify themselves and to what extent and those new organs or the ones that might undergo histolysis during the process of development.

Further Athalia proxima is an important crop pest, as its larval forms are destructive to the foliage of cruciferous plants. They are voracious feeders. It has been observed that within days a highly infested field may be completely eaten up, leaving bare stem of the plant. Such attacks are usually followed by migration of larvae to the adjoining fields where food is abundantly available. They feed on radish, turnip, mustard and cauliflower but radish and mustard are preferred.

The Embryology of Athalia proxima has been done by Farooqi (1963) who has given a fairly complete account of the early development of the egg and details of organogeny. Dhillon (1966) has worked out the morphology of this insect and has given a short account of its histology in immature stage. The present work deals with the post-embryonic development of this insect with particular reference to the histological changes that the digestive system undergoes in various larval instar. During the course of investigations on the first instar larva it was found that soon after hatching it starts feeding on the foliage of the host plant. This food being vastly different from the one on which the embryo subsists. It was thus found desireable to study the structure of the alimentary canal of the embryo also. Microscopic slides of embryos about to hatch were made available to the present writer and a detailed study of the embryonic structures was

made possible. The nutritive requirements of the embryo are met with by the stored deutoplasm which is gradually absorbed by the midgut cells. The dissolution of the yolk having been brought about by the vitellophages which are reported to disappear during the late embryonic life (Farooqi, 1963). It was anticipated that a sudden change of food and food habits is likely to affect the histological feature of the alimentary canal specially the mesenteron. Study of such changes was considered to be of great significance. Certain points of some consequence in this behalf were correlated with the change of the food in first instar larva.

II. REVIEW OF LITERATURE

Considerable work has been done on the descriptive embryology of the order Hymenoptera, specially the sub-order Apocrita. The honey-bee has frequently been used in the study of normal and experimental embryology. Nelson (1915) has given an account of the embryology and post-embryology of the honey-bee. Oertel (1930) has described its metamorphosis. In case of symphyta, which is a primitive sub-order of the order Hymenoptera, there are some papers on the embryonic development. Graber (1890) described briefly the morphogenesis of Arge berberidis (Hylotoma berberidis of Graber , Argidae). Shafiq (1954) has given a short account of the embryology of Pteronidea ribesii (Tenthredinidae). Ando & Okada (1958) have described the external form of the butter stem sawfly, Aglaostigma occipitosa. Their interest is confined mainly to a comparative study of the embryonic development of the two sub-orders of Hymenoptera and to compare the embryology of symphyta with that of Lepidoptera.

Enough work has been done on the biology of sawflies. Miles (1936) has described the external features of the larval forms and the biology of Emphytus cinctus, and Blennocampa salicaimi. Carleton (1938) gave the biology of Pontania proxima the bean-gall sawfly on willows. Smith (1970) has

described the biology of three genera of Tenthredinidae in the sub-family Nematinae (Phyllocalpa, Pontania and Emura). Pflugfelder (1934) described the embryonic and post-embryonic development of the silk glands of Pontania salicis (Tenthridinidae).

A fairly exhaustive account of the embryology of Indian mustard sawfly, Athalia proxima has been given by Farooqi (1963). Another important work on this insect is that of Dhillon (1966), who has described the morphology of the adult and only a brief account of a fully grown larva.

The development of the alimentary canal during the post embryonic development has attracted the attention of several workers. Nelson (1915) gave only a brief account of the structures in the alimentary canal of the larva of the honey bee. Various structures in the last larval instar of the honey-bee have been described by Oertel (1930).

Some important comprehensive works on post-embryology of Lepidoptera are also available in the literature which provide source material for a comperative study with the Hymenoptera. Henson (1929) confined his study to the development of midgut in the larval stages of Vanessa urticae. He gave details of all the structures in the midgut and their successive development from the first to the fifth instar larva. In his next paper, Henson (1931) has taken the entire

alimentary canal of the same insect and described the post-embryonic changes in the various larval instars. Later, Henson (1932) also described the development of the alimentary canal in Pieris brassicae and advocated the endodermal origin of the malpighian tubules. The work of Judy and Gilbert (1970) deals with the histology of the alimentary canal of the last instar larva of Hyalophora cecropia and its metamorphosis and structural changes in gut at larval-pupal transformation and pupal - adult transformation.

Ganguly (1959) has given an account of the histology and anatomy of the alimentary canal of the larva of Bolitophila luminosa of New Zealand.

III. MATERIAL AND TECHNIQUE

The larvae of mustard sawfly, Athalia proxima are abundantly found in the post-monsoon months on radish and mustard plants. These were collected from the field during the month of October and were brought to laboratory for rearing. The larvae collected belonged to different instars. They were placed in separate tubes measuring 3" x 1". These tubes were half filled with sand for the purpose of pupation. The larvae were fed on fresh radish leaves. The fifth instar larva pupates in the sand already provided for this purpose. After two or three days pupae which have developed a cocoon were sorted out and placed in the rearing cages. The cocoon is silken with a silvery shining inner surface and encrusted with sand particles on the outer. Such cocoons can be easily removed from the sand mass without damaging the young pupa. The adult emerges and feeds on sugar soaked in cotton. Two changes of food were given in 24 hours. After copulation the female deposits the eggs singly forming a neat row along the margin of host plant leaf. After hatching the young larvae were transferred to other tubes where they were fed on radish leaves. Each moult was counted and each instar was labelled according the number of moults cast off from the different larvae. Different instars were selected and taken out from the tubes when desired.

The larvae were killed in hot water. At about 60°-65°C temperature it was found that the larvae curl slightly but soon straighten out and cause no difficulty in subsequent manipulations. They were then placed in Bouin's fixative after making slight incision to ensure complete penetration. The material remained in Bouin's fixative for 20 hours. The excess of picric acid was removed by using 70% alcohol. The material was dehydrated in graded series of alcohol. Larvae were transferred from 100% alcohol to a 50-50 mixture of absolute alcohol and Benzene and then in pure Benzene. After complete clearing the material was then transferred to a 50-50 mixture of clearing agent and paraffin wax (60°-62°C) and left in an oven maintained at 65°C for one hour. It was then placed in pure infiltration medium and back to oven. This was repeated twice over the next one hour. Then it was removed from the oven and oriented in blocks. Sections were cut at 5 and 6 microns and fixed to slides with the help of albumen glycerin. Streched sections were dried at 50°C in the oven for 24 hours.

Several stains were tried on the material but the best results were obtained with Delafield's hematoxylin either alone or in combination with eosin. 5% iron alum was used as a mordant and slides were stained in hematoxylin at 55°C for half an hour. Differentiation was done in .1% acid alcohol. Stained sections were cleared in clove oil and mounted in dilute canada balsam.

IV. BIOLOGICAL OBSERVATIONS

The larva of Athalia proxima resembles the Lepidopterous caterpillar. Its soft skin is lighter in color in the first instar but gradually turns olive green in the last one. The hypognathus dark black head is prominently displayed. The longitudinal axis of the head is inclined at an angel of about 120^{0} to the long axis of the body. The skin of the larva is not smooth but presents a wrinkled appearance giving a false impression of annulation. The trunk is divided into 13 segments, three thoracic and ten in abdominal region. Their external grooves are well seen in longitudinal section of both embryo and larva. There are ten pairs of spiracles which are situated dorso-laterally on both sides of trunk. The first two spiracles are situated on thoracic region while the rest are on the abdominal segments. There are three pairs of thoracic legs and seven pairs of prolegs from 2-8 abdominal segments. The first abdominal pair forms the pleuropodia as reported by Farooqi (1963) which according to him grows into a conical structure and disappears altogether before the close of embryonic life.

There are five larval instars. These take 14 to 17 days to complete their life under favourable conditions of temperature. As the temperature falls in the months of

December they take longer time to moult. Before moulting the color of the larva is dark olive green but soon after moulting it appears lighter. Sometimes it is transparent to such an extent that the movement of the waste pallets can be seen easily through the body wall. As the newly moulted instar ages it becomes darker and darker especially in the later instars.

The first instar larva measures about 2-3 mm in length and .5 mm in diameter. It is light olive green in color. Its larval duration is about 3 days. The newly moulted second instar is about 5-6 mm long and its diameter is about 1 mm. Its duration is also 3 days. The third instar after moulting, on an average, is about 10 mm in length and its diameter is 1.5 mm. This instar takes longest time to moult into the next one and its duration ranges 4-5 days. The fourth instar after moulting measures 13-14 mm in length and 2 mm in diameter. This instar takes 2-3 days to moult. The fifth instar which is the last one varies from 15-16 mm in length and 2 mm in diameter. Its larval duration varies considerably even under the same conditions of temperature and humidity. Its duration varies ranging from 24 hours to 72 hours and then it goes to pupate. The larval life on the whole lasts for about 14 to 17 days.

Observations made on feeding habits of the first instar larva show that usually it starts feeding from the underside

on the green portion of the leaf leaving the thin epidermic on the opposite side intact. A small circular hole is thus caused on the under surface of the leaf. This habit is similar to other sawflies notably the cladius spp.& Endelomyia aethiops and Emphytus cinctus as reported by Miles (1936). After every moult their appetite seems to increase and they consume more and more leaves. The third and fourth instars are voracious feeders. Dhillon (1966) has reported that the fourth and fifth instar larvae are voracious feeders. The observations of the present writer go to suggest that the fifth instar larva takes no food at all. The ravages of the larvae are enormous, eating away most of the green portion of the leaves causing considerable damage to the standing crops. Sometimes the larvae stay motionless but when disturbed they curl up and fall down on the ground. They later wander about before reaching the host plant again.

The larvae move upwards when they begin to moult leaving behind the cast skin on the tips of the leaves in the field and on the covering cloth in the rearing jars.

V. HISTOLOGICAL CHANGES IN THE ALIMENTARY CANAL

1. Alimentary canal of the embryo before hatching:

The alimentary canal in late embryo is a simple canal divided according to its embryonic origin into stomodaeum, midgut and proctodaeum.

(a) Stomodaeum

The origin and early development of stomodaeum in the embryo of mustard sawfly is given by Farooqi (1963). It originates in the form of an ectodermal invagination with its blind end directed obliquely backwards. As the growth progresses it extends up to the junction of the head with the trunk - a condition which is observed in an embryo about to hatch. The oesophagus, the proventriculus and the salivary glands have their origin from the stomodaeum. Towards the posterior side of the stomodaeum the wall inflects inwards at certain points so as to give it a folded appearance to the otherwise circular lumen. The tube is surrounded by a closely set single layer of epithelial cells with rounded nuclei. The intima lining these epithelial cells is very thin but distinct. The epithelial cells are surrounded by muscle cells which are indistinctly arranged into longitudinal and circular fibres.

The oesophageal valve is well established and clearly recognisable. This valve is formed by the invagination of posterior portion of stomodaeum into the anterior region of midgut. It is made up of two arms, one which is going downwards and after making a loop in the anterior most part of the midgut, it moves upwards forming its second arm when it reaches upwards to a point where midgut starts it joins the later and this is the place where head and trunk join each other. Both arms of the valve are made up of ectodermal cells similar in form as described above in stomodaeum. A thin limiting membrane separates the stomodaeum from the midgut. It is made up of very small cells with small rounded nuclei. These cells are flattend. It is very thin in the middle and probably this is the weakest point where it breaks in later stages forming a passage between the stomodaeum and midgut.

(b) <u>Midgut:</u>

The midgut in mustard sawfly is bipolar in origin (Farooqi, 1963). It originates from anterior and posterior midgut rudiments. The stomodaeum and proctodaeum push these rudiments to a more internal position and finally meet each other and enclose the yolk completely. The cells of the midgut undergo several changes before they attain definite form. In the late embryo tall vacuolated cells appear and pseudopodia - like outgrowths are formed from their free ends as

reported by Farooqi (1963). A considerable amount of yolk has been used up and only a few scattered spherules can be observed in the gut region. According to him these pseudopodia like outgrowths of the midgut cells help in engulfing the yolk spherules.

In the late embryo midgut is a simple canal with some yolk spherules. The epithelial cells are tall, separated by the cell walls which are not very clear. There is no differentiation between different types of midgut cells, namely columnar cells and regenerative cells as reported by Dhillon (1966) in the adult of A. proxima nor is there yet any trace of brush border which is characteristic of midgut cells of the larva, and the adult. The nuclei are rounded and the cells surrounded by muscles cells still do not show a clear distinction between longitudinal and circular fibres. The lumen of midgut is almost uniform throughout its length. The midgut cells measure 5 to 6 microns in length. Towards the anterior end of the midgut there is seen the oesophageal valve with a thin limiting membrane separating the stomodael region from the midgut. Likewise, at the blind end of the proctodaeum there is again a thin limiting membrane separating the former from the midgut. These membranes later rupture to allow free communication of the stomadael and proctodael parts with the midgut.

(c) Proctodaeum:

Usually the proctodaeum originates as a posterior ectodermal invagination but in A. proxima Farooqi (1963) reports that it originates in an unusual way, contributed both by a portion of the amnion and the germ band. The invaginated posterior end carries all its way the amnion which extends as a continuous membrane on the ventral side of the embryo. Finally, at the time when the larval form is recognizable, it becomes parallel to the long axis. Towards its anterior blind end a thin limiting membrane is present which arises by the thinning out of cells of the proctodael wall and is, therefore, considered as ectodermal in origin. It shuts off the hindgut from the midgut but late in the embryonic life it ruptures to allow free communication between the two.

Proctodaeum in late embryo is a short tube occupying the posterior portion of the trunk. Definite structures of the hindgut which are easily recognizable in the first instar larva are not fully differentiated, yet a close study of these sections reveals that some differentiation in the cellular arrangement is in the offing.

The anterior portion is a wide funnel like structure constricted a little posteriorly. This part will give rise to the pylorie region. The constricted portion behind represents the pylorie valve. In a cross-section the lumen

is hexagonal and the epithelium is 2-3 cell layers thick at six points. This is the region which will form the pyloric valve. Beyond it the lumen expands a little and again constricts posteriorly at the place where rectal valve will develop. This region in cross-section is two layers in thickness. The region between the two constricted portions may be termed as anterior intestine.

The posterior most part of the proctodæum is an expanded tube which can be designated as rectum. Its cells are simple with rounded nuclei. Intima is quite thin. A thin coat of muscle cells surrounds the whole proctodæum without differentiating into circular and longitudinal muscles. This coat is stronger around the two constricted valvular regions especially the anterior one.

Posteriorly, the rectum opens through a broad opening which is directed anteriorly since the embryo lies folded upon itself so as to bring the head close to the anal opening.

2. Alimentary canal of the larvae:

(a) Foregut:

There appears to be no remarkable change in position of the foregut in the late embryo and the first instar larva. It passes backward from the mouth and joins the midgut at the level where the head and trunk join together. Nelson (1915) reports the same condition in the first instar larva of the honey-bee. The anterior portion of the foregut in a young larva in cross-section appears a little laterally flattened (Fig.-1, Plate I), while its posterior portion forms four longitudinal folds extending upto the oesophageal valve (Fig.-2). These are formed by infolding of the wall of the foregut into its lumen. The lumen for the same reason appears cross-shaped in a transverse section. These folds are not very deeply inflected in the first instar. Nelson (1915) described similar folds in the honey-bee. The epithelial cells forming the wall of the foregut are cuboid with small rounded nuclei. They measure 2.7 microns with 1 micron nuclei. The intima is thin but quite distinct. Circular and longitudinal muscles are present. The muscular coat is well developed at the place where the foregut forms the oesophageal valve. Ganguly (1956) in the larva of Bolitophila luminosa (Diptera) has reported that the muscular coat just in front of the oesophageal valve is about three times thicker than the muscular layer of the anterior region of the oesophagus.

The oesophageal valve is formed by the invagination of oesophageal wall for a short length into the lumen of the midgut, then is reflected back to join the anterior end of the midgut (Fig.-3). It is usually described as a cylindrical fold of the foregut projecting into the lumen of the midgut. The oesophageal valve is composed of three layers in a cross section. The inner layer is slightly folded. The middle layer is the outer reflected layer of the oesophagus and the outermost layer is the layer of epithelial cells of the midgut (Fig.-4). Ganguly (1956) reported the same condition in B. luminosa. The cells which form the two arms of the oesophageal valve are also cubical in shape with rounded nuclei.

In the second instar there is slight elongation of the foregut. The size of the cells and nuclei increases slightly. The cells measure 8 microns and nuclei 5 microns in diameter. The oesophageal valve becomes more distinct.

No remarkable change in the foregut of third instar is visible. Its size however, increases a little as compared with the first and second instars. The cells measure 10 u and the diameter of nuclei is about 7 u.

In the fourth instar it attains a considerable size extending a little beyond the union of the head with the trunk. The lumen of the foregut is narrow but the folds further deepen and in a cross-section present a stellate appearance.

The epithelial cells measure 13 microns, the nuclei 7 microns in diameter. The intima becomes quite thick. The oesophageal valve proceeds deeper into the anterior midgut region. The two arms of the valve move farther apart from each other after each moult. The muscular coat also becomes much stronger in the fourth instar as compared to earlier ones.

Up to the fourth instar the only change observed in the foregut is an increase in size of the cells, the nuclei and the intima. In the last instar, the fifth one the changes undergone are quite remarkable which may be conceivably regarded as preliminary to metamorphosis. As the fifth instar larva ceases to feed and empties its alimentary canal, the foregut is reduced to a narrow tube surrounded by a poorly defined muscularis. The individual cells constituting these muscles do not exhibit their definitive cellular form. The walls are less prominent and the nuclei stain darker. Oertel (1930) reported in case of honey-bee that the foregut in the larva about to seal is a short structure about 1 to 1.5 mm in length and nuclei of the cells are dark staining. The epithelial folds of the foregut further deepen reducing further its lumen. In a cross-section the lumen appears roughly X-shaped with long arms (Fig.-5, Plate II). The nuclei of the epithelial cells are quite large and distinct. The cells measure roughly 13-14 microns in length and nuclei 7-8 microns in diameter. The intima covering the epithelial cells remains

no more smooth and presents a serrated appearance. Further changes in the intima shall be reported in the prepupal stage. Judy and Gilbert (1970) reported in Hyleophora cecropia that the chitinous intima in the foregut of fifth instar separates from epithelial cells.

The oesophageal valve constricts and withdrawns itself a little. Judy and Gilbert (1970) reported the same condition in the fifth instar larva of H. cecropia at the time of pupal moult.

(b) Midgut:

The midgut is the longest portion of the gut occupying most of the trunk region. It extends from the junction of the head and trunk up to the seventh trunk segment. It has the form of a long, almost straight tube of more or less uniform diameter. Anteriorly it communicates with the oesophagus through oesophageal valve. The epithelium has a basement membrane on which rest two kinds of cells - the columnar cells and the regenerative cells.

The columnar cells are the main representative of midgut epithelium. They are tall cells with centrally placed rounded or elongated nuclei. They lie on basement membrane and possess brush border (striated border) towards the lumen of the gut.

The regenerative cells are small cells found inbetween the tall columnar cells in groups of 5-8 cells. In early instars they are not visible but become distinct as the larval age advances. They are round cells with small rounded nuclei. A thin peritrophic membrane is present in the midgut region around the food mass. It is less differentiated in the first instar. It is observed that sometimes it inflects inwards inbetween the food masses for a short length. The membrane is in the form of a close bag and projects a little in the anterior portion of the hindgut at the union of the mid- and the hindgut (Fig.-7).

Both circular and longitudinal muscle layers are present around the midgut. Here the circular fibres underlie the longitudinal ones.

First instar - In the first instar the midgut is a relatively large and straight tube. No regenerative cells are observed at this stage. The epithelium consists of cells about 10-13 microns long carrying a continuous striated border measuring 1.5 microns and nuclei about 4 microns in diameter. The nucleus has the form of a chromatic mass immersed in a large vacuole. Hertig (1923) described a similar state in living gut cells of the adult honey-bee. The lumen of the midgut in the middle part measures 135 u in diameter. Many columnar cells have globular projections directed into the lumen which in the embryo have been described as pseudopodia - like

outgrowths of the epithelial cells. These globule like projections are observed only in the first instar larva of A. proxima (Fig.-8 & 9, Plate III). No such projections are seen in any other instar at any stage. These projections though observed throughout the midgut but seem to be more numerous in the anterior and posterior regions.

As regards the function of these projections there is some differences of opinion among the workers. Henson (1929) observed such globular projections in the second instar in the midgut of Vanessa urticae and regarded them as disintegrating cell pieces. Van Gehuchten (1890) working on Ptychoptera regarded them as gut secretion. Later Deegener (1909) and Bordas (1911) described them as secretary granules in Deilephila euphorbiae. In A. proxima their existance in the late embryos and the first instar larvae may be associated with the change in the quality of food during the transition from the embryonic to larval life. It may be recalled that the embryo is totally dependent on deutoplasm for its nutritive requirements. The yolk cells which are abundantly found in the deutoplasm during embryonic life are supposed to dissolve the yolk so that it becomes easily assimeable by the embryo. The pseudopodia - like projections in the midgut region are claimed to engulf the food matter and also increase the absorptive surface of the epithelium. Soon after hatching the food abruptly changes from protein rich (Gressom, 1929) to carbohydrate rich food - a

change of some consequence for the physiological makeup of the larva. The newly emerged larva has to consume leafy material and the physiology of digestion therefore is likely to be altered. The form of the epithelial cells is suggestive of the secretary nature of these cells. There is, however, no evidence of cells undergoing any disintegration as suggested by Henson (1929) in the midgut of Vanessa urticae. Another suggestion is that these globules in the first instar larva may be meant for the formation of peritrophic membrane. Demerec (1950) has described that small globules or a more or less continuous layer of refringent substance are present in the cardia of the imago of Drosophila melanogaster. He concludes that presumably it is a formative stage of the peritrophic membrane.

Second instar - The mesenteron of the second instar has the same features as in the first excepting the disappearance of the globular bodies mentioned above. The midgut is approximately 300 u in diameter, roughly an increase of about one third. The regenerative cells are yet not distinguishable. The columnar cells are 13-24 microns tall with nuclei 8 microns in diameter and with a brush border 2 microns high.

Third instar - In case of third instar the gut lumen measures about 500 microns or 5 mm in diameter. The chief components of the midgut epithelium are columnar cells, but there seems

to be some indication of the appearance of regenerative cells whose number does not exceeds more than 2-3 cells per cross-section. The columnar epithelial cells present a retangular shape measuring 40 to 59 microns with ovoid nuclei measuring 12 microns. Towards the lumen side the ends of the epithelial cells have a brush border which forms a clear, less staining continuous zone in which the striations are not distinct under the highest power of the light microscope.

Fourth instar - In the fourth instar there is not much change as compared to third one except a little increase in the size of cells. In the region of the oesophageal valve the anterior portion of the midgut enlarges to form a cup-shaped structure whose basal part constricts and then expands to form the regular midgut region (Fig.-10). The columnar cells start elongating so as to assume a taller appearance. Their length varies from 37 to 72 microns and nuclei measure 15 microns in diameter. The nuclei are oval and centrally placed. The cells possess 4-5 microns high brush border. Both muscle layers are distinctly visible around the midgut epithelium. The circular muscles lie towards the inner side surrounded by longitudinal fibres.

Fifth instar - Unlike the various instars described above the fifth instar exhibits important changes in the midgut as a prelude to pupation. In the present study the larva of the fifth instar has been sectioned in the early stage, middle,

and in the stage just before pupation. The chief points of interest are discussed below.

It has already been mentioned that the fifth instar larva stops feeding altogether. In the early fifth instar the regenerative cells become distinct and appear at the bases of the epithelial cells. They increase in number and continue to do so in the later stages. It is at this stage that the columnar cells begin to disorganize and this process progressively increases in the later stages.

Midway in the life of the fifth instar the columnar cells and their nuclei elongate considerably. The cell walls become less distinguishable. They measure roughly 62 to 143 microns in length and the long axis of the nuclei measure 18 to 20 microns. At the bases of some of these cells, groups of regenerative cells are distinctly visible and become more conspicuous. Judy and Gilbert (1970) report a similar condition in the fifth instar larva of Hylophora cecropia. These groups of cells consist of about 8-12 small rounded cells measuring 8 microns with nuclei 2.7 microns in diameter (Fig.-11). Their number continues to increase till the larva passes into the pre-pupal stage. These cells stain heavily in hematoxylin stains, and stand out sharply from the adjoining cells of the epithelium. In each cross-section 2-3 such groups of regenerative cells are visible at this stage. The columnar cells begin to detach at their bases and are pushed into the lumen

of the gut. Such groups of cast-off cells are abundant in the posterior region of the midgut especially near the junction of midgut with the hindgut (Fig.-12, Plate IV). The cells in the anterior region of the gut are still in contact with their basement membrane (Fig.-13). This indicates that the process of sloughing off of the old epithelium starts from posterior region and proceeds anteriad.

Later, before the fifth instar passes into the pre-pupal stage the regenerative cells become active and produce a fresh epithelium which is neatly laid down under the older one (Fig.-14). Henson (1929) reports a similar condition in the late fifth instar larva of Vanessa urticae. Unlike the older epithelium the freshly produced epithelium has small cuboid cells with rounded nuclei set in a distinct row. Each cell measures 10 microns. The freshly formed epithelium stains lightly as compared to the older one. No striated border has yet made its appearance. Simultaneously with the formation of the fresh epithelium the older one is cast off. The cells detach themselves from the gut, loose their identity and appear like a crumpled mass in the lumen of the midgut. Vacuoles appear in these masses and are probably digested during the prepupal life.

Mention has already been made above of the accumulation of the rejected epithelial cells in the posterior part of the midgut which gives an impression of a plug blocking the passage

into the hindgut. In the honey-bee Oertel (1930) has reported that a tissue plug makes its appearance after the larva is sealed. This plug is formed by the epithelial cells of the adjacent portion of the ventriculus and closes its passage between the midgut and the hindgut. He states that probably this plug prevents the entrance of any material from the midgut into the hindgut for the period during which the young imaginal cells in the later are undergoing division and are not protected by a chitinous intima. In case of A. proxima it could not be ascertained whether a real plug develops or not. Work along these lines is in progress.

(c) Hindgut:

The hindgut is the posterior most part of the main three gut divisions. It is a short tube as compared to the midgut and occupies the last three trunk segments. It is further divided into different regions which differ from each other structurally. Anteriorly it joins the posterior most part of the midgut and opens outside through the anus at the posterior end of the body. The hindgut is differentiated into the pylorus, the anterior intestine and the rectum or posterior intestine. The nomenclature adopted here is the one suggested by Snodgrass (1935).

Pylorus - The pyloric region is a short wide funnel - like tube leading back from the anterior most part of the hindgut up to the pyloric valve (Fig.-15, Plate V). The union between

the midgut and the pylorus is marked by a small fold of epithelial wall directed inwards. The pylorus recieves malpighian tubules anteriorly just behind this fold. Posteriorly the pylorus constricts to form the pyloric valve. The inner walls of the valve are thrown into six folds which are quite distinct in a cross-section (Fig.-16). This valve closely resembles the structure found in Vanessa urticae which Henson (1931) named as anterior sphincter region. In A. proxima the pylorus in cross-section looks like a circular ring surrounded by a narrow layer of epithelial cells and a thin musculature. The cells of this region are flattened with small nuclei. The intima is quite smooth. Transverse muscles and an outer layer of longitudinal fibres is present. Unlike the pylorus the pyloric valve is surrounded by strong muscles. Both muscle layers are present but the circular fibres are stronger than the longitudinal ones, since the former are disposed in several layers while the later consists of a single layer. The epithelial cells lining the pyloric valve stain darkly. The intima in this region is thick and shows distinct serrations which gives it an uneven appearance.

Anterior intestine - The part of the intestine lying between the pyloric and the rectal valves is the anterior intestine (Fig.-15). In a cross-section the lumen of this part is not entirely circular but the wall seems to have developed slight foldings. The broad tubular part of the anterior intestine

is made up of cubical cells with rounded nuclei. Both layers of muscles are present around the epithelium. Henson (1931) called this region as the colon in Vanessa urticae.

Rectum or posterior intestine:- The union of anterior intestine with the rectum is marked by the presence of a valve called the rectal valve (Fig.-17). It resembles the pyloric valve in structure. In cross-section it appears folded but there is no regularity in the arrangement of these folds, unlike those in the pyloric valve where six distinct folds are present. The cells and their nuclei stain heavily with hematoxylin. The muscular coat around this region is stronger.

The rectum is a laterally expanded structure divisible into two regions, anterior and posterior on the basis of its structure. The whole rectum is surrounded by circular muscles inside and the longitudinal ones on the outer side.

The anterior rectum forms the first half of rectal chamber. The wall of this region is so folded so as to form long finger-like projections projecting into its lumen (Fig.-18). Wigglesworth (1932) considers that they are sites at which water is conserved by resorption from the faeces, and inorganic ions may also be resorbed through them. The epithelium of these projections is composed of large cells with no distinct boundaries and contains darkly stained nuclei occupying most

of the portion of the cells. The intima over this region presents the same serrated form as in the pyloric valve region. Its musculature is week.

The posterior rectum is in the form of an open funnel opening posteriorly through the anus. It differs from the anterior rectum structurally. Its epithelium is made up of cubical cells which are arranged in a single row. The intima is thin and smooth. The cells lining the posterior rectum closely resemble those seen in the adjacent body wall of the larva. The anus is the posterior opening of the gut situated at the end of the last trunk segment. The waste products pass out from this opening which is supplied with muscles.

Malpighian tubules - In a transverse section the wall of the Malpighian tubules consists of 3-4 epithelial cells enclosing a circular lumen. They open into the hindgut at the junction of the midgut with the former (Fig.-19, Plate VI). The cells constituting the wall of the Malpighian tubules possess cilia towards their inner borders which project into the lumen.

The origin of the Malpighian tubules is a matter of some controversy. Nelson (1915) in honey-bee regarded them to be of ectodermal origin, but Henson (1932) described its functional part originating from endoderm in the case of Pieris brassicae. In the gooseberry sawfly Pteronidea ribesii (Shafiq, 1954) they are considered to have originated from

ectoderm. Later Poulson (1950) confirmed Henson's view. Savage (1956) reported a similar observation in case of Schistocerca gregaria. Srivastava and Bahadur (1961) while working on Dysdercus koenigi described Henson's view to be highly probable. In the embryo of Athalia proxima (Farooqi, 1963) these tubules are regarded to have originated from ectoderm. In the larvae of A. proxima they open into a neutral zone at the site where the mid- and hindgut unite (Fig.-19), as indicated by Wigglesworth (1939). If they are considered to be a part of hind intestine there ought to be present an intima which is lacking here. On the other hand the presence of striated border is suggestive of its resemblence to the midgut. Based on this evidence it may be argued that the functional part of the tubule is derived from the midgut as suggested by Henson (1932) in P. brassicae and in D. melanogaster by Strasburger (1932). And yet there may be another possibility of their ectodermal origin as embryonic observations go to suggest and that the ciliated epithelium is a secondary development in response to the function assigned to them.

All the structures mentioned above are present in the first instar larva. In the second instar the hindgut does not exhibit any marked change except in size. The cells of the pylorus are extremely thin measuring 4 microns in thickness. The cells of pyloric valve on the other hand increase a little

and measure 7 microns in length. The epithelial cells of rectal valve and rectum measure 8 microns and 6 microns respectively.

In the case of third instar the epithelial cells of pylorus measure 6 microns and pyloric valve 7 microns, while the cells forming anterior intestine measure 5-4 microns. Folds of rectal valve become much deeper and its cells measure 13 microns while those of rectum measure 8 microns.

In the fourth instar, the pyloric region becomes a little longer measuring 89 u in diameter. The anterior intestine shortens in length and appears as a bulbous chamber instead of a tubular structure seen earlier (Fig.-20). Due to this change in form the valvular regions come a little closer to each other. The diameter of the anterior intestinal region in the middle measures about 169 microns. The cells of the pylorus measures 6 microns and pyloric valve 7 microns. The epithelial cells forming the anterior intestine measure 6 microns. The rectal valve and rectum measure 14 microns and 9 microns respectively.

Before passing into the prepupal stage the fifth instar larva however, undergoes some remarkable changes. The regional differentiation in the hindgut is no more distinct and it can now been divided into an anterior and a posterior part (rectum) (Fig.-21). This condition is retained in the

adult as described by Dhillon (1966). The two valvular regions cease to be as distinct as in the previous four instars. The muscular coat which was much developed in the region of pyloric and rectal valves is no more clear, although an aggregation of muscle fibres is seen in these regions including the anterior intestine. The intima is quite thick and light staining. The epithelial cells became irregular. Cell walls are not distinct. Nuclei are large. Cells and their nuclei stain lighter.

The rectal walls become highly folded. The intima is thick and seems loosely attached to epithelial cells. The cells show no distinct boundries and there is no evidence of any sloughing off of the hindgut cells yet. At this time an imaginal ring is observed at the junction of midgut with the hindgut (Fig.-22, Plate VII). The ring is composed of small cells having one or two nuclei.

In a late fifth instar just before pupation in the vicinity of imaginal ring new cells are proliferated which in a cross section form a continuous ring (Fig.-23). Each cell measures about 10 microns. The larval epithelium of the hindgut become compact but it is not cast off yet (Fig.-24).

VI. SUMMARY

The olive green caterpillar - like larva of Athalia proxima has a distinct head and a trunk divided into 13 segments. There are ten pairs of spiracles, 3 pairs of thoracic legs and 7 pairs of prolegs. The five larval instars have a duration of 14 - 17 days. The first instar starts feeding soon after hatching from the underside of the leaf. Third and fourth instars are voracious feeders while the fifth does not feed at all.

The alimentary canal in the late embryo is a simple canal with a short stomodaeum extending up to the head capsule. Oesophageal valve is distinct. A delicate limiting membrane separates the stomodaeum from the midgut. The midgut is long and tubular with no trace of striated border. The proctodaeum is a short tube occupying the last three trunk segments. Various regions of the hindgut are not easily recognizable.

In the larva, the lumen of the oesophagus is X-shaped in a transverse section. The oesophageal valve is a prominent structure with a cylindrical fold projecting into the anterior region of the midgut. Before pupation the fifth instar exhibits remarkable changes.

The midgut in the larva extends from the junction of the head and trunk up to the 7th trunk segment. The

regenerative cells, clearly visible in the fifth instar, are present in groups inbetween the tall columnar cells. In the first instar larva many columnar cells have globular projections directed into the lumen of the midgut. In the fifth instar larva the columnar cells disorganize progressively and the regenerative cells form a new epithelium beneath the older one.

The hindgut is differentiated into different parts structurally. The anterior most part is the pylorus with the pyloric valve at its posterior end. The next following is the thin - walled anterior intestine which is connected to the rectum through the rectal valve. The two valvular regions are folded structures. The rectum is divisible into an anterior and a posterior part which opens outside through anus.

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PLATES

PLATE I

EXPLANATION OF FIGURES

Fig. 1. Transverse section of the head of first instar larva showing phrynx. P, Phrynx. X 200.

Fig. 2. Transverse section of oesophagus of the fifth instar. E, epithelium; CM, circular muscles; LM, longitudinal muscles. X 250.

Fig. 3. Longitudinal section of the oesophageal valve of first instar. ME, midgut epithelium; OT, outer limb of the valve; IL, inner limb ; OE, oesophagus. X 400.

Fig. 4. Transverse section of oesophageal valve of the first instar. ME, midgut epithelium; OV, oesophageal valve; M, muscles. X 160.

PLATE-I

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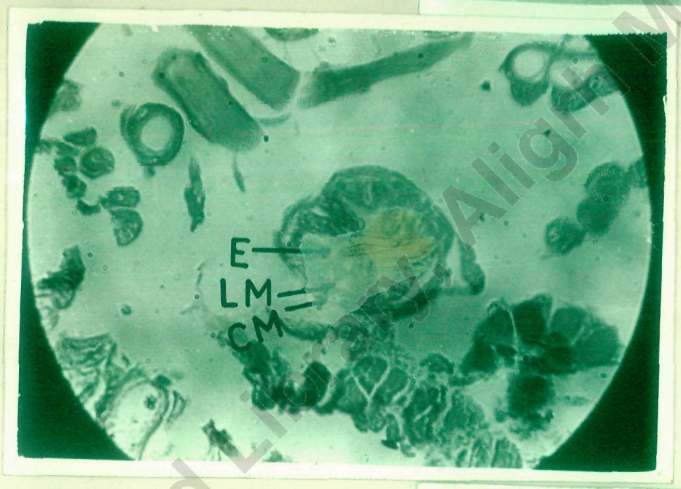

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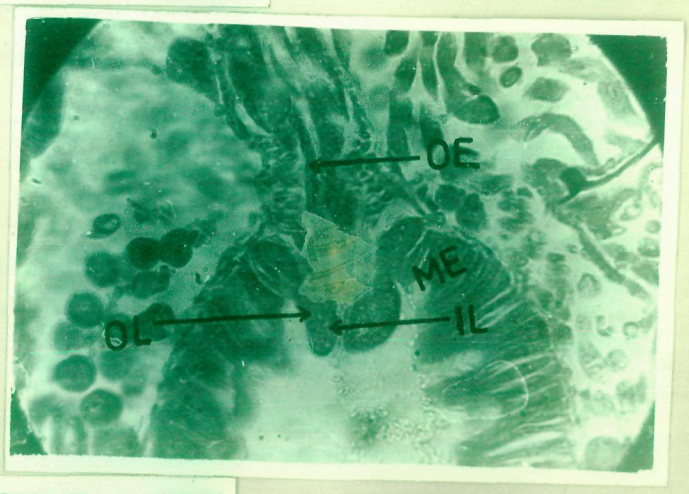

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PLATE II

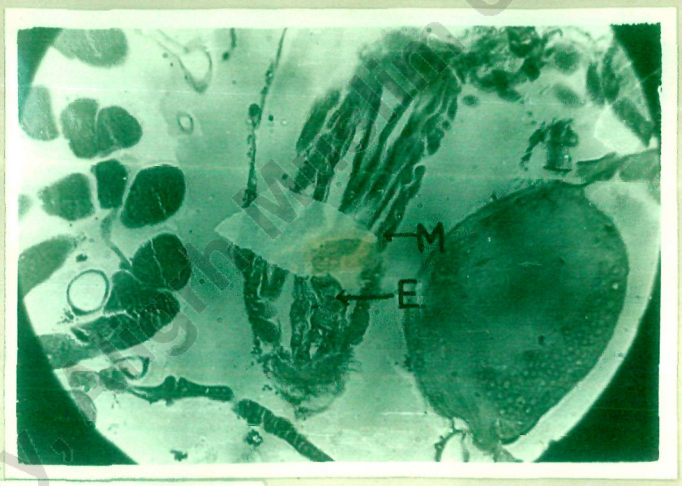
EXPLANATION OF FIGURES

Fig. 5. Transverse section of oesophagus of the fifth instar. E, epithelium; M, muscles. X 160.

Fig. 6. Longitudinal section of oesophageal valve of the fifth instar. ME, midgut epithelium; OV, oesophageal valve. X 160.

Fig. 7. Longitudinal section of the third instar showing peritrophic membrane. PM, peritrophic membrane. X 160.

PLATE - II



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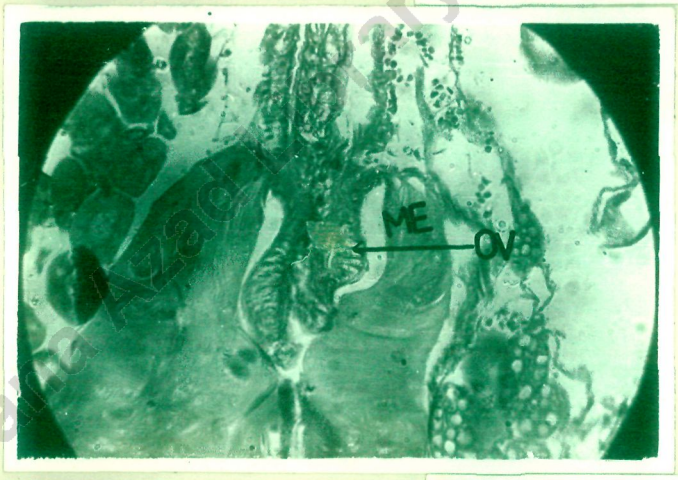

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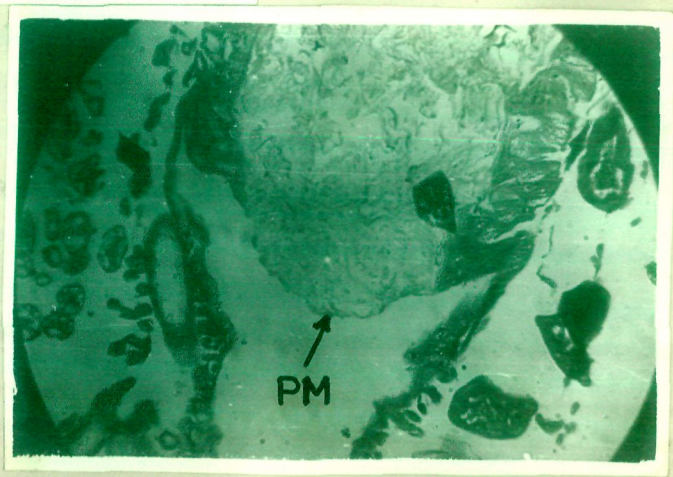

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PLATE III

EXPLANATION OF FIGURES

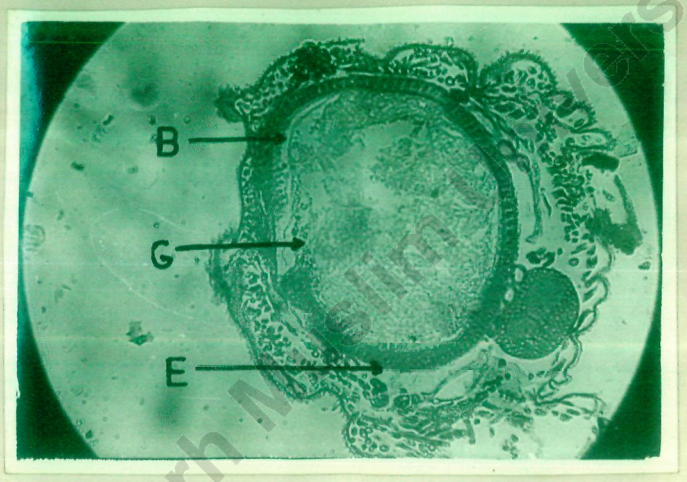
Fig. 8. Trans verse section of the midgut of the first instar. G, globules; B, brush border; E, epithelium. X 160.

Fig. 9. Longitudinal section of midgut of the first instar showing globular projections. G, globules; E, epithelium. X 250.

Fig. 10. Longitudinal section of the anterior region of midgut of the fourth instar. ME, midgut epithelium; OV, oesophageal valve. X 160.

Fig. 11. Transverse section of midgut of the middle stage fifth instar showing groups of regenerative cells. RC, regenerative cells; E, epithelium. X 160.

PLATE - III



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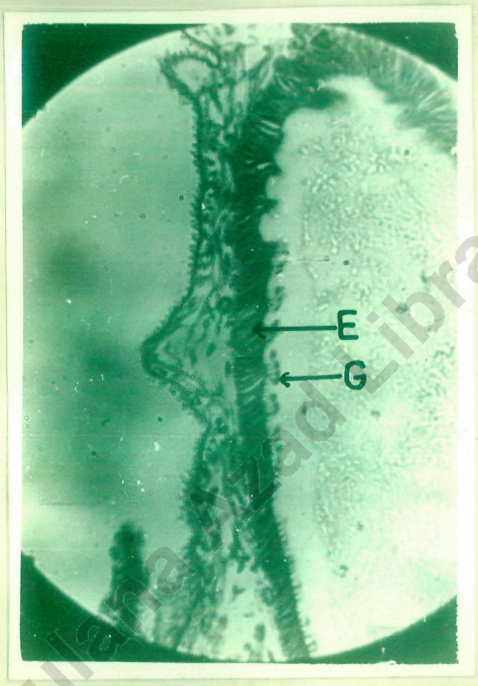

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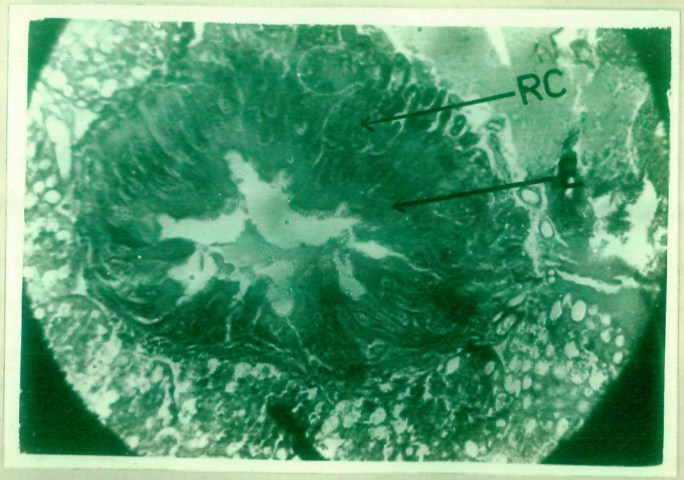

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PLATE IV

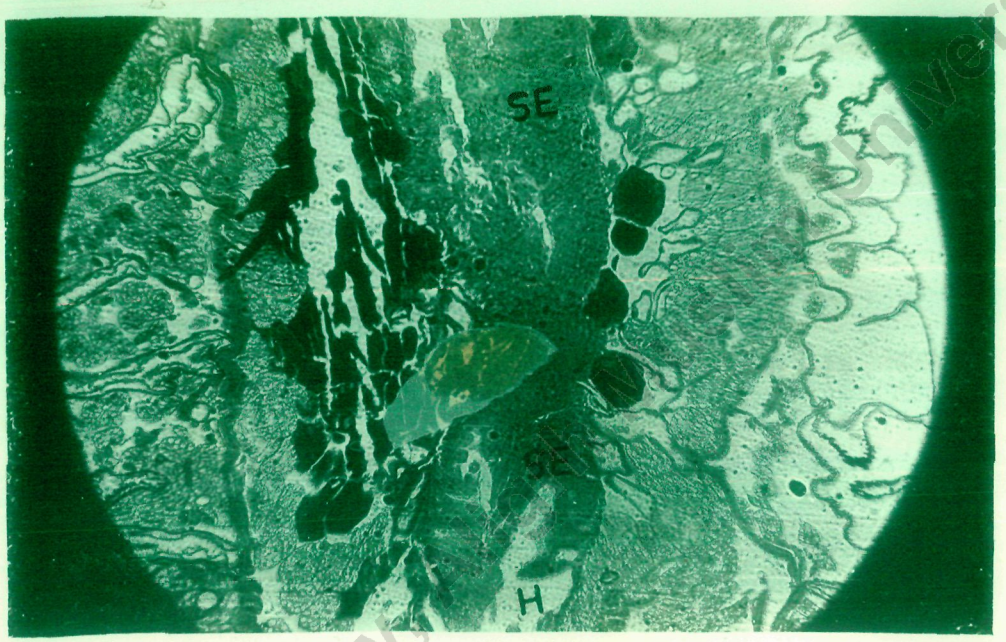
EXPLANATION OF FIGURES

Fig. 12. Longitudinal section of the middle stage fifth instar through the posterior midgut region showing sloughed off epithelium. SE, sloughed off epithelium; H, hindgut. X 200.

Fig. 13. Longitudinal section of the middle stage fifth instar showing infact epithelium of the anterior region of midgut. E, epithelium; L, lumen. X 160.

Fig. 14. Transverse section of midgut of the late fifth instar. OE, older epithelium; NE, new epithelium; M, muscles. X 160.

PLATE - IV



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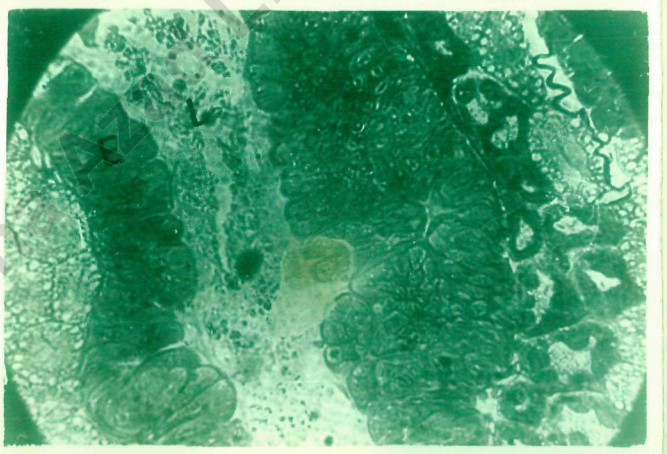

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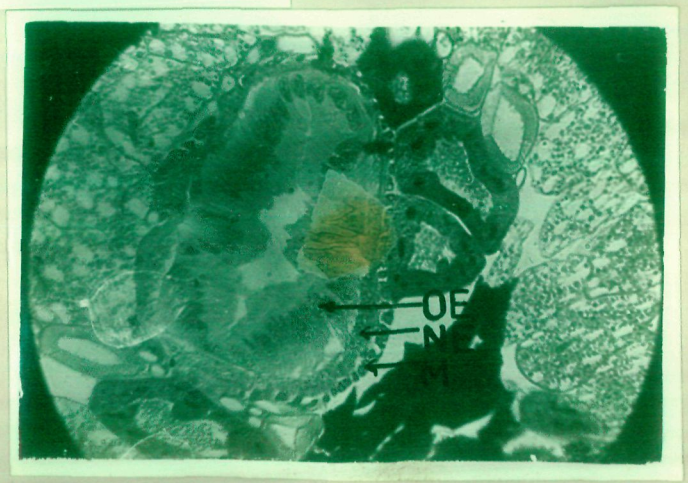

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PLATE V

EXPLANATION OF FIGURES

Fig. 15. Longitudinal section of hindgut of first instar. E, midgut epithelium; M, midgut lumen; PY, pylorus; PV, pyloric valve; AI, anterior intestine; RV, rectal valve; R, rectum. X 160.

Fig. 16. Transverse section of the hindgut of first instar through pyloric valve. E, epithelial fold; LM, longitudinal muscles; CM, circular muscles. X 250.

Fig. 17. Transverse section of the hindgut through rectal valve. RV, rectal valve; CM, circular muscles. X 160.

Fig. 18. Longitudinal section of the rectum of the first instar larva. AR, anterior rectum; PR, posterior rectum. X 250.

PLATE-V

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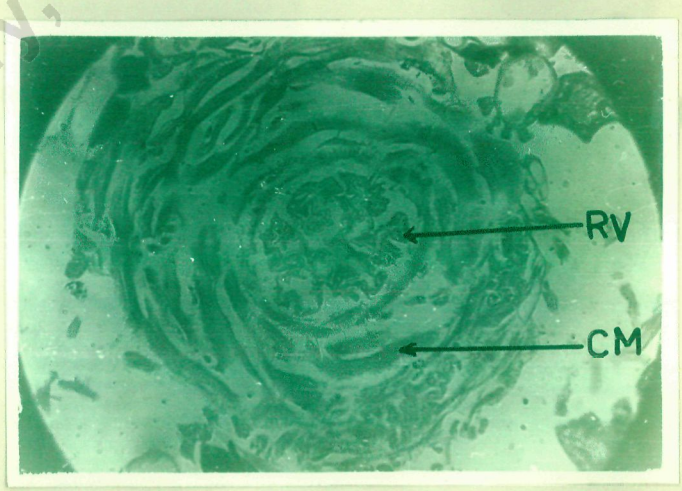

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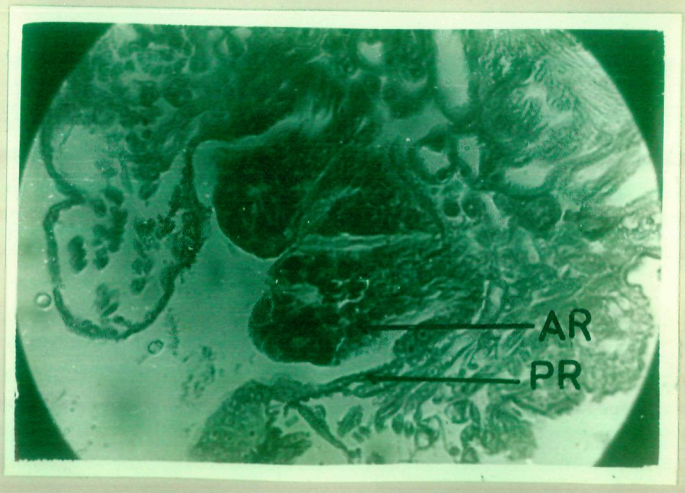

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PLATE VI

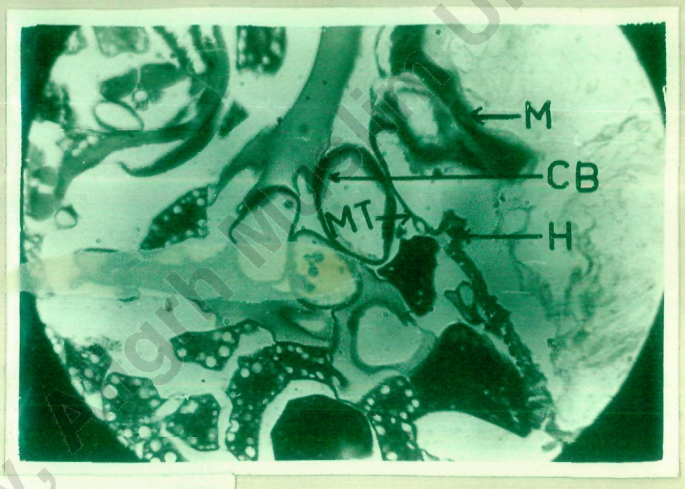
EXPLANATION OF FIGURES

Fig. 19. Longitudinal section of the fourth instar larva showing Malpighian tubules and the opening. M, midgut epithelium; CB, ciliated border; MT, Malpighian tubules; H, hindgut epithelium. X 160.

Fig. 20. Longitudinal section of the fourth instar larva showing anterior intestine. AI, anterior intestine. X 100.

Fig. 21. Longitudinal section of the hindgut of middle stage fifth instar. PY, pylorus; PV, pyloric valve; AI, anterior intestine; RV, rectal valve; R, rectum. X 100.

PLATE-VI



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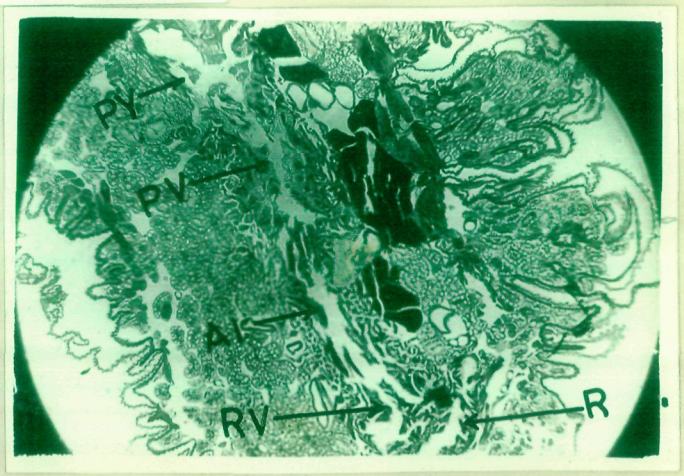

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PLATE VII

EXPLANATION OF FIGURES

Fig. 22. Longitudinal section of the middle stage fifth instar showing imaginal ring. M, midgut region; IR, imaginal ring; H, hindgut epithelium. X 250.

Fig. 23. Transverse section of the late fifth instar showing imaginal ring. ME, midgut epithelium; IR, imaginal ring. X 160.

Fig. 24. Transverse section (oblique) of late fifth instar. REF, rectal epithelial folds. X 160.

PLATE-VII

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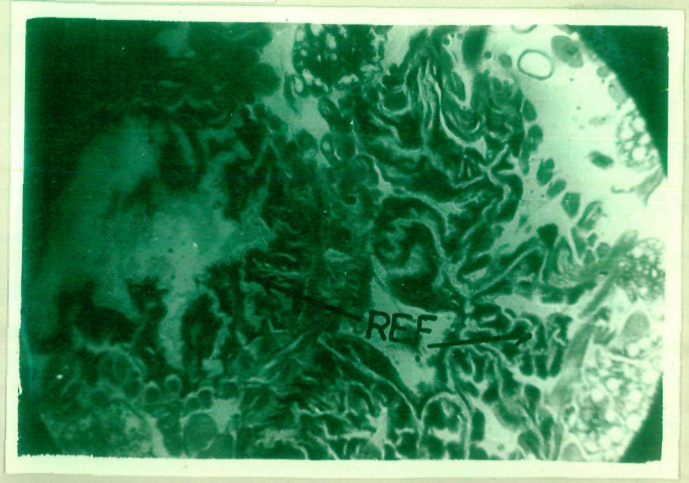

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